

Using Next-Generation Sequencing to Accelerate Blight-Resistance Improvement and Investigate Ecologically Important Traits in Indiana's American Chestnut Breeding Program

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Introduction

Despite its location on the edge of the natural range of American chestnut (*Castanea dentata*), Indiana developed one of the earliest American chestnut breeding programs after The American Chestnut Foundation (TACF) began backcrossing hybrids of American chestnut to the resistant Chinese chestnut (*Castanea mollissima*) in the early 1980s. The goal of the program is to develop blight-resistant chestnuts that fulfill the currently empty niche of American chestnut in the central hardwood forest. At several locations around Indiana, third-backcross (BC3) seed orchards of individuals selected for high blight resistance and *dentata*-like features are producing seed. Their offspring, BC3F2 trees, are currently being screened and selected as parents for the BC3F3 generation, which should display consistently high levels of blight resistance and American chestnut-like form. We present a plan for my dissertation research, which will use next-generation DNA sequencing to examine the genetic basis of blight resistance in Indiana's breeding program, and evaluate the potential use of DNA markers to make early selections and speed up the selection process. I will also map quantitative trait loci (QTL) for traits related to seed dispersal (such as seed size) in the BC3F2 generation of trees. These ecologically important traits are important to understand because they will govern the relationship between restored chestnut and native seed dispersers, and are interesting from an evolutionary standpoint because of the coevolution of mast-bearing trees in the Fagaceae with mammalian and avian seed dispersers.



Figure 4. BC3 seed orchard at Jackson-Washington State Forest.

Figure 2 (below left). Stem girdling caused by chestnut blight cankers on American chestnut.
Figure 3 (below right). Size comparison of American chestnut seed, top, and Chinese chestnut, bottom. Seed size is variable in hybrid and backcross offspring.



Figure 1. Schematic of TACF's American chestnut backcross breeding program assuming 3 major blight resistance loci on 3 chromosomes (Kubisiak et al. 2013).

- 1. Initial hybridization.** Performed shortly after blight's introduction, in the 1920s. Blight resistance alleles in Chinese chestnut are indicated with black. Assume that only trees with Chinese resistance alleles are advanced at each subsequent step.
- 2. First backcross.** Performed shortly after step 1. "Graves" and "Clapper," the two individuals used as donor (resistant) parents in the first 15 years of the TACF program, were both BC1 trees from a USDA breeding program that ended in the 1960s.
- 3. Second backcross.** The BC2 pollen parent of Indiana's current breeding population was a BC2 tree from Meadowview, VA (TACF) planted in the 1980s. It was part of the first series of backcross made by TACF in the 1980s. "Clapper" pollen was used to pollinate a surviving American chestnut mother in VA.
- 4. Third backcross.** In Indiana, BC2 pollen was put onto a number of American chestnuts growing in the state.
- 5. BC3 Intercross.** Crucially, this is the first cross whose offspring have a chance of inheriting two recombinant chromosomes with blight resistance from Chinese chestnut.
- 6. Selection of BC3F2.** Most BC3F2s will have some combination of blight resistance alleles, but the best will be true-breeding (homozygous) at all three loci. These are selected to form seed orchards. A weakness of the Indiana breeding program is that the two alleles will be identical-by-descent at each locus, making resistance less resilient.

Project Objectives

Marker-assisted chestnut breeding

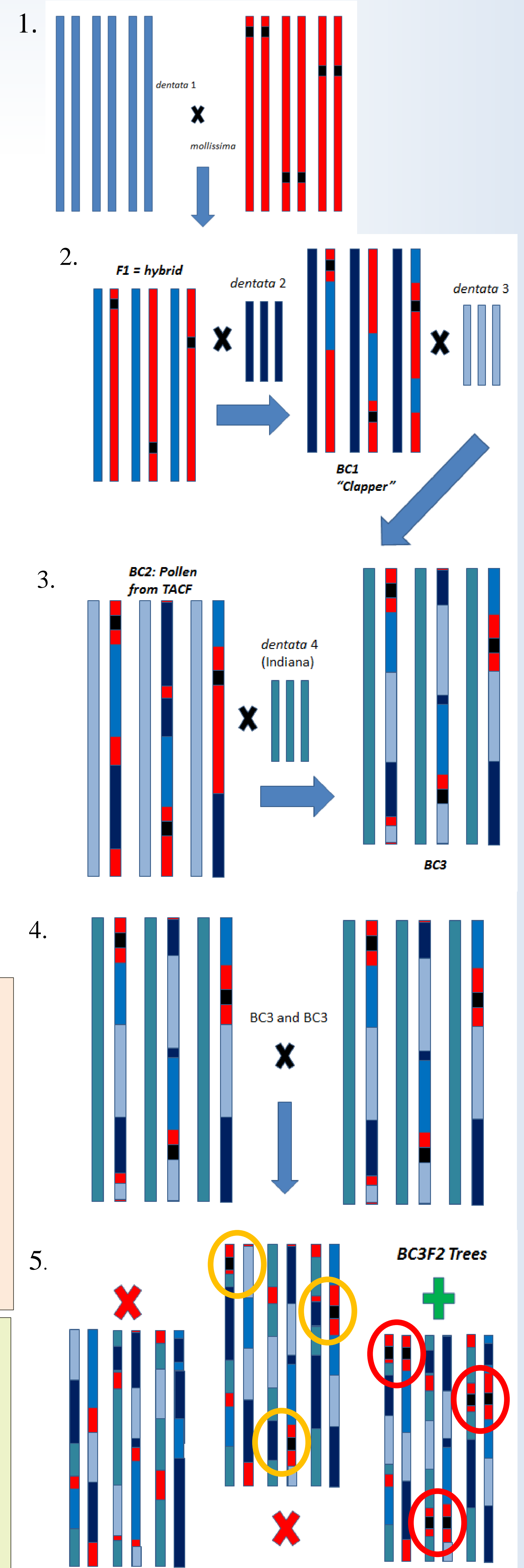
- Map blight resistance quantitative trait loci (QTL) in the Indiana breeding population using a large number of single-nucleotide DNA markers (~5K) derived from genotyping-by-sequencing (GBS).
- Use whole-genome regression to make phenotype predictions; assess accuracy of this technique for making early selections.

Mapping ecological traits in hybrid trees

- Using GBS-derived markers, map QTL for seed-dispersal and survival traits
- Validate these QTL in multiple years
- Correlate QTL related to squirrel dispersal behavior with those for seed nutritional and size traits

Assessing the range of variability and gene diversity in blight-resistant Chinese chestnut germplasm

- Investigate the diversity of blight resistance haplotypes within 10 individuals of a diverse germplasm collection of Chinese chestnut (*Castanea mollissima*) using whole-genome-resequencing.
- Use resequencing data to examine the seed trait QTL within Chinese chestnut, identifying whether or not seed traits have undergone strong recent selection in Chinese chestnut due to human cultivation.



References

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- Baird, N.A. et al. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLOS ONE* 10.1371/journal.pone.0003376.
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Acknowledgements

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Tree Material

- A large trial of BC3F2 sapling trees growing at the Southern Indiana Purdue Agricultural Center (SIPAC) will be used for blight resistance QTL mapping and development of marker-assisted selection models.
- BC3F2 seed from the Jackson-Washington State Forest (JWSF) in southern Indiana will be used for seed trait QTL mapping.
- Chinese chestnut selections to be resequenced will be drawn from the diverse collection at Empire Chestnut Company in Carrollton, Ohio.

Methods

- A new leaf-inoculation method will be tested on the SIPAC BC3F2 population, with conventional stem inoculations to test the accuracy of the leaf technique. These inoculations will start in Summer 2014.
- DNA will be extracted from seeds prior to dispersal trials using a few milligrams of cotyledon tissue. A hole needs to be punched in the seeds to insert a wire tag (Xiao et al. 2013), so waste material will be used to extract DNA.
- Dispersal trials will take place in the field October-December for three consecutive years (2014-2016). Populations of squirrels accustomed to chestnut (in and around seed orchards and test plantings) will be used to measure the differences in dispersal distance and consumption behavior among American, Chinese and hybrid chestnut and within BC3F2 hybrid seeds.
- RAD-seq genotyping-by-sequencing (Baird et al. 2008), with 100-bp Illumina paired-end reads and samples multiplexed 96/lane, will be used to derive genotypes for QTL mapping.
- A detached-branch inoculation assay will be used to test resistance of pure Chinese chestnuts.
- Illumina sequencing, with two barcoded individuals per lane, will be used to resequence Chinese chestnut (genome size ~770 Mbp).